

## SOME AUSTRALIAN ANAPORRHUTINE TREMATODES.

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Early in the present year three species of *Probolitrema*, *P. rotundatum*, *P. clelandi* and *P. simile*, were described from South Australian clasmobranchs (Johnston, 1934). The last two, from the same host, were stated to be closely related and perhaps synonyms. Since additional material of these species has become available for study, the opportunity was taken to re-examine the late Professor S. J. Johnston's type slides of *Petalodistomum cymatodes* and *P. polycladum*, for which thanks are tendered to Professor Harvey Sutton of the School of Public Health and Tropical Medicine, University of Sydney.

## STAPHYLORCHIS CYMATODES (Johnston) Travassos.

This species, originally described as *Petalodistomum cymatodes* by S. J. Johnston (1913, 392), was based on a single specimen collected from the body cavity of a spotted stingray (leopard ray), *Dasyatis kuhlii* M. & H., from Townsville, North Queensland.

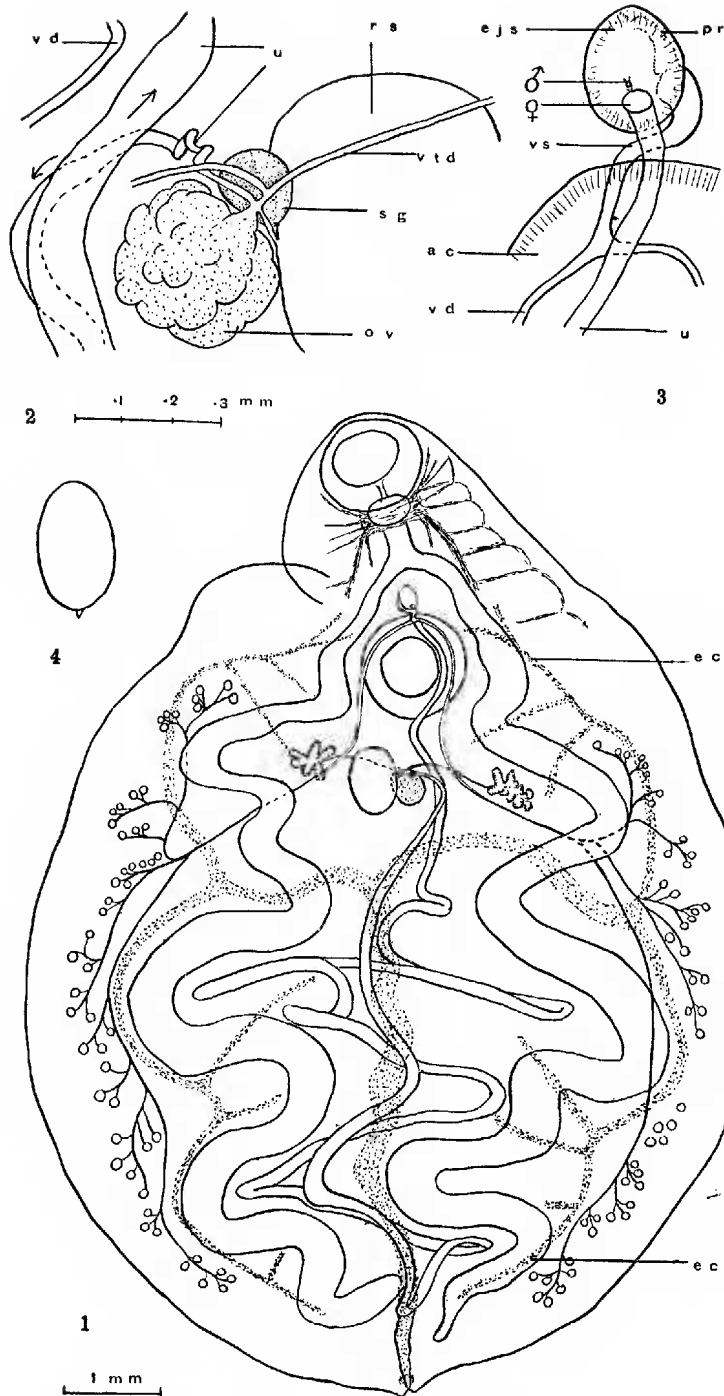
Mainly on account of the characters of the testes and intestine, Travassos (1922, 226) erected the genus *Staphylorchis* for it, giving the following diagnosis:—Anaporrhutinae; body flat, broad; pharynx present; intestinal crura sinuous, without diverticula; vitellaria intracaecal; testes extracaecal, very small and numerous. He regarded it as being nearer to *Anaporrhutum* than to *Petalodistomum*. Fuhrmann (1928, 112) referred to the genus as *Straphylorchis*.

Thanks to the kindness of Professor Harvey Sutton, School of Public Health and Tropical Hygiene, University of Sydney, the type slide was made available for further study. The form and general anatomy are shown in Johnston's figure, but a closer examination of the specimen has permitted an amended and more detailed account, especially of the reproductive and excretory systems.

*S. cymatodes* is a large trematode, 10.4 mm. long by 1.7 mm. broad, with the suckers approximately equal in diameter (1.1 mm.). The genital pore lies a short distance in front of the acetabulum. The pharynx is somewhat lens-like, 0.50 mm. broad by 0.31 mm. long. There is a rather wide oesophagus. The crura are at first narrow, gradually widening, being broadest in their posterior half. They are thrown into a number of wide undulations and terminate near the end of the worm.

The specimen has the brain and main nerves arising from it well shown. The brain has the usual position above the pharynx and from it are given off, more or less symmetrically, nerves passing forwards along the anterior sucker, several short ones laterally and a long dorsal and ventral from each posterior corner, the ventral being the larger. The latter travels posteriorly in close association with the intestine. It gives off numerous lateral nerves which all become connected with a lateral nerve near the margin of the parasite. The dorsals were not traced backwards.

The main excretory canals were traceable in the specimen. The vesicle is tubular and extends a great deal further forward than the original figure indicates. A little distance behind the ovary this longitudinal canal bifurcates, each branch passing outwardly to meet the corresponding lateral vessel in the vicinity of the mid-length of the worm. The connections shown in the original figure, between the posterior end of each lateral canal and the vesicle, are incorrect. The course



Figs. 1-4.

*Staphylorchis cymatodes*.—1, Type, dorsal; 2, female complex, ventral; 3, region of genital pore, ventral; 4, egg. a c, acetabulum; e c, excretory canal; e j s, ejaculatory sac; o v, ovary; p r, prostate; r s, receptaculum seminis; s g, shell gland; t, testis; u, uterus; v d, vas deferens; v s, vesicula seminalis; v t d, vitelline duct.

of the tubes arising from the lateral vessels is indicated in fig. 1. From the anterior branch, one is given off to the vitelline region, one to the area just in front of it, and the bifurcate end drains the part between the suckers. The posterior branch is the larger and from it arise two tubes near one another, and a smaller one further back. The main excretory ducts are thus disposed more like those of *Anaporrhutum*.

The type specimen has 47 round testes on one side and 52 on the other. They lie extracrually extending from just in front of the level of the vitellaria to a point a short distance in front of the end of the crura. They are about 0.1 mm. in diameter. The arrangement of the ducts shows that though the vesicles appear to form a continuous series there are really three groups of them on each side, a small anterior group of 13 to 17, a middle group of about 12 to 15, and a posterior group comprising the remainder. Each vesicle is continuous with a delicate duct and groups of two to four of these efferent tubules join to enter the larger ducts. The anterior vas deferens passes backwards close to the crus (and may lie below a loop of it) to join the middle posterior vas a short distance behind the level of the ovary. The posterior vas is the largest duct and may or may not underlie a lobe of the crus. It travels forwards near the excretory canal, its anterior part lying inwardly from, and approximately parallel to, the middle vas. It meets the combined anterior and middle ducts, and then passes inwards below the intestine and forwards diagonally just inwardly from the corresponding yolk gland. At the level of the ovary it bends forwards, travelling above the acetabulum and inwardly from the crura. The two vasa met above the anterior margin of the ventral sucker, to form a small vesicula seminalis. This travels forwards near the midline above the metraterm and ejaculatory sac, and enters the latter in its anterior region. The walls of the sac are thickened, probably due to the presence of prostate glands. The structure is about 0.28 mm. long by 0.2 mm. broad. The male aperture is adjacent to the uterine pore, but the exact relationships were not made out. The arrangement appears to be very like that figured by Looss (1902, pl. 23, fig. 32) for *Plesiochorus cymbiformis*, except that the vesicula is quite small.

The ovary is median, a little distance behind the ventral sucker. It measures about 0.32 mm. broad by 0.32 mm. long and has a number of low, broad, rounded projections, the organ appearing rather compact. The short, wide oviduct travels forwards and inwards and, after receiving the tiny duct from the receptaculum, enters the shell gland which lies in front of it in an angle made by the receptaculum and the anterior border of the ovary. The seminal receptacle is rounded, 0.53 mm. long by 0.67 mm. wide, lying slightly to one side of the midline. The shell gland measures 0.15 by 0.18 mm. The fertilizing duct, after traversing it, becomes thrown into a few very short convolutions immediately in front of the ovary and then passes, as the uterus, below the descending limb of that organ. Its course is thus different from that previously described, and is similar to that in other Anaporrhutine trematodes. It now follows a more or less median course until it reaches the region between the ends of the crura. It then becomes thrown into a few wide loops in the post-ovarian part of the body, the loops extending almost from one crus to the other. It eventually passes forwards above the early portion of the duct, then to one side of, and close to, the ovary and forwards above the acetabulum to terminate just in front of it and just behind the cirrus sac. Very few eggs are present in the type, and most of them are very distorted. A typical specimen measured 0.05 by 0.028 mm. and possessed a small terminal projection. The largest egg observed measured 0.065 by 0.040 mm., excluding the "spine." The projection varied considerably in length, being most marked in under-sized eggs. S. J. Johnston gave 0.06 to 0.064 by 0.03 mm. as the dimensions. The yolk glands lie internally to the crura and

very slightly in front of the level of the ovary. Each consists of a branched tube, the branches being short and rounded and clustered at the end of the yolk duct. The two ducts pass almost directly inwards just in front of the ovary, one of them lying ventrally to the anterior region of the receptaculum. They join below the shell gland, and a very short, common duct passes back to unite with the fertilizing duct just after its junction with the canal from the receptaculum and immediately before it enters the shell gland.

Our study of the type indicates that *Staphylorchis* is more closely related to *Anaporrhutum* than to any of the other genera in the subfamily, but differs from it in the extracaecal distribution of the minute spherical testes; the completely intercaecal position of the vitellaria; and the sinuous form of the intestinal crura. In the last of these characters, as well as in the form of the excretory vesicle and of the testes, it differs from *Petalodistomum*, which also has a branching intestine.

In 1930 Nagaty republished Johnston's figures (in part) and gave a generic diagnosis evidently based on Travassos. He also reviewed the Anaporrhutinae, giving a tabulated comparison of the chief anatomical features of the genera assigned to the subfamily.

Baylis (1927, 426) transferred *Anaporrhutum largum* Luehe, 1906, to *Staphylorchis*, and gave a figure of a specimen from *Stegostoma tigrinum* from Madras. Luehe's material came from the coelome of a Cingalese ray, *Rhinoptera javanica*. Southwell (1913, 101) recorded it as *A. largum* from *Chiloscyllium indicum*, *Ginglymostoma concolor* and *Aetobatis narinari* from Ceylon, and mentioned that a species of *Anaporrhutum* was taken from *Stegostoma tigrinum* off Orissa. He referred to certain differences between the specimens from these two localities, but thought that they probably belonged to the same species.

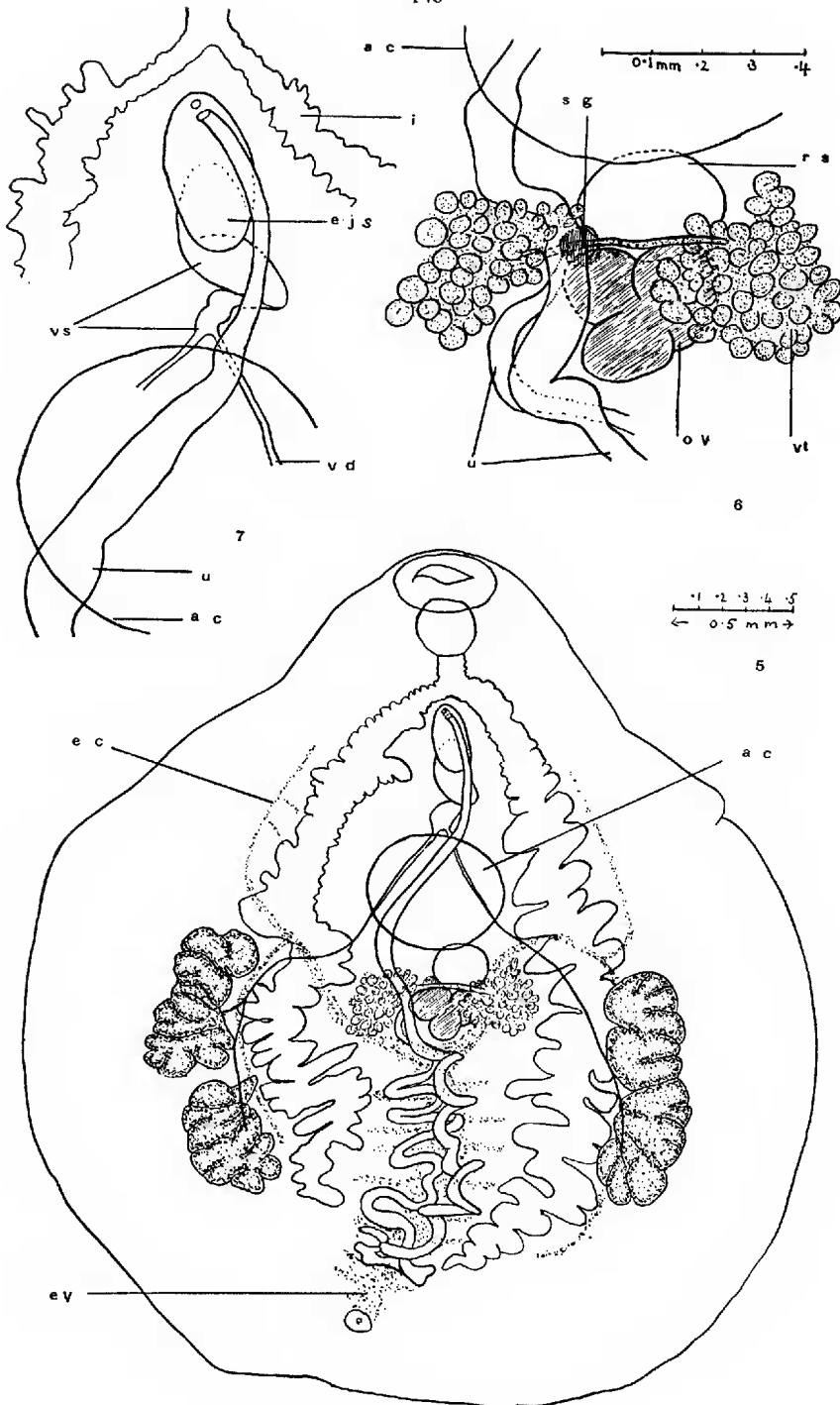
Nagaty (1930, 102) gave a brief account of *S. largus* from *Ginglymostoma concolor* from Ceylon. Some of his specimens showed amphitypy, as he stated that the ovary and metraterm occurred on either the right or the left of the midline. He mentioned that the excretory vesicle was Y-shaped.

Judging from Baylis' account, *S. largus* is more circular in outline; it has larger and fewer testes, and these are much more restricted in distribution; the lateral regions of the body are much more extensive; the intestinal folds are much closer, more numerous and less regular; the uterine folds occupy a much more restricted region, and there is a marked difference in the sizes of the two suckers, the ventral being  $2\frac{1}{2}$  times the oral in diameter. The testes, though few in number, were found by Baylis to be unequal on opposite sides, just as Luehe had observed. A similar condition occurs in the type specimen of *S. cymatodes*.

#### PETALODISTOMUM POLYCLADUM S. J. Johnston.

This species was obtained from the body cavity of *Dasyatis kuhlii* M. & H., from North Queensland (S. J. Johnston, 1913, 389-92). Thanks to the kindness of Professor Harvey Sutton, the type slide and a series of sections were made available for further study. Unfortunately the series is somewhat incomplete and disarranged and obviously made from a distorted specimen. These defects have prevented a more satisfactory account of the excretory system being given. The type is small and almost round, 3.76 mm. long by 3.5 mm. broad, narrowing somewhat in the anterior third, but with a broadly rounded posterior region. S. J. Johnston (1913, 390) reported that the length of his specimens varied between 3.3 and 3.7 mm., and the breadth between 3 and 3.5 mm. The type must have been his largest specimen.

The oral sucker has a diameter of 0.44 mm. and the ventral 0.65, the ratio being 1 : 1.47, the original account stating that the oral ranged from 0.375 to 0.424 and the ventral from 0.636 to 0.652 mm., the ratio being 1 : 1.6. The



Figs. 5-7.

*Petalodistomum polycladum*.—5, Type, ventral, position of male ducts and excretory system added from serial sections; 6, female complex—ventral; 7, region of sex apertures, ventral, composite figure from type and sections. Figs. 6 and 7 drawn to same magnification. a c, acetabulum; e c, excretory canal; e j s, ejaculatory sac; e v, excretory vesicle; i, intestine; o v, ovary; r s, receptaculum seminis; s g, shell gland; u, uterus; v d, vas deferens; v s, vesicula seminalis; v t, vitellarium.

pharynx is 0.25 mm. in diameter and is succeeded by a short oesophagus. The crura which terminate some distance from the posterior end, possess numerous short, irregular sacculations or short, wide branches given off from both the inner and outer surfaces. These irregularities are also present, though small, in the region of the bifurcation also. The intestine is remote from the lateral edges of the worm.

The serial sections do not allow of information additional to that in the original account being given regarding the nervous system. The excretory pore is definitely dorsal, about midway between the posterior limit of the uterus and the end of the parasite. The vesicle extends forwards above the uterus to the hind margin of the ovary, then bifurcating, the limbs extending outwards and forwards above the vitellaria, one limb being adjacent to the outer edge of the seminal receptacle. They then pass below the intestine and, at about the level of the rear edge of the acetabulum, each branches into a shorter anterior and a larger posterior excretory canal lying immediately laterally from the intestine. The posterior canal occupies a position between the latter and the corresponding testis. The point of origin and the course of the ducts entering the lateral canals could not be determined satisfactorily from the material under examination. The main vesicle is not a simple tube but, as Johnston has stated, possesses a number of lateral projections.

The testis of each side lies laterally from the intestine and close beside it. Each extends forwards practically to the mid-length of the worm, and thus lies behind the level of the posterior end of the acetabulum. The posterior fifth of the body is free from them. Each testis forms one or two masses and presents a much lobulated surface, the lobes being short, broad, and closely set. The testicular ducts from the anterior and posterior portions of the gland lie near the inner margin. They join near the middle of the organ to form the vas deferens which travels forwards and inwards below the intestine at about the level of the receptaculum. The vasa pass close to the inner side of the corresponding caecum, one vas lying between the latter and the metraterm. They meet in the midline just above the anterior edge of the acetabulum and form a swollen seminal vesicle which becomes thrown into a few short convolutions lying above the metraterm. The ejaculatory sac is rather small (about 0.3 mm. long by 0.15 mm. broad) and lies below and in front of the anterior end of the vesicula. Its walls are considerably thickened owing to the presence of gland cells. There is a narrow canal leading from the sac downwards to the male pore which is situated immediately in front of the uterine aperture, the two lying just behind the intestinal bifurcation.

The ovary has three rounded lobes, two anterior and one posterior. It measures 0.32 mm. in maximum breadth by 0.28 mm. in length, and is about 0.8 mm. in diameter. It lies on the right of the midline above the right anterior ovarian lobe, and is in close contact with the rounded receptaculum. The latter, which measures 0.29 mm. in breadth and 0.22 mm. in length is situated just to the left of the midline immediately in front of the ovary and partly overlaps it. It lies adjacent to the posterior margin of the acetabulum. Its extremely narrow duct arises posteriorly and travels inwards between the ovary and receptaculum, joining the short oviduct very close to the ovary. The fertilizing duct passes diagonally forwards through the shell gland, then becomes slightly twisted and widened and passes back directly above the descending limb of the uterus. The two limbs keep rather close company and occupy a restricted median region, which extends posteriorly a little beyond the ends of the intestine, but does not reach the crura laterally. The descending limb passes forwards to the right of the ovary, which it may partly underlie, below the shell gland and to the right of the receptaculum. In the type it passes below the yolk duct but above some of

the right vitelline lobes. It then travels above the acetabulum, becoming median. Its terminal portion, the metraterm, was seen, in section, to be thrown into numerous small ridges. This region passes below one of the vasa deferentia, the vesicula and the ejaculatory sac. The female pore lies immediately behind the male aperture, the two being confluent. As S. J. Johnston states, eggs measure 0.052 to 0.063 mm. by 0.023.

The vitellaria occupy a restricted region intercrurally on either side of the ovary. Each has about forty or fifty small rounded projections in the type, though S. J. Johnston mentioned that the gland was composed of 15 to 20 small rounded follicles. Nagaty (1930, 104), in his tabulation of the main characters of the species, states that the vitellarium consists of "small spherical follicles." They are really short tubular processes. Some of those belonging to the left gland underlie part of the ovary and receptaculum. The transverse yolk duct lies ventrally immediately in front of the ovary and underlies part of the receptaculum and the shell gland. A common duct passes upwards in a curved course to lie between the shell gland and the ovary, joining the fertilizing duct in the vicinity of the former.

S. J. Johnston's original generic diagnosis included *Petalodistomum cymatodes* which Travassos removed to a new genus, *Staphylorchis*. Nagaty (1930, 106), in his review of the Anaporrhutine trematode genera, amended slightly Johnston's diagnosis. Our examination of the type does not necessitate any further modification. The generic characters may be stated briefly thus:—Anaporrhutinae; intestinal caeca with numerous sacculations; testes lobed, extracrucial; vitellaria composed of numerous tubular lobes, intercrural; excretory vesicle with numerous lateral diverticula.

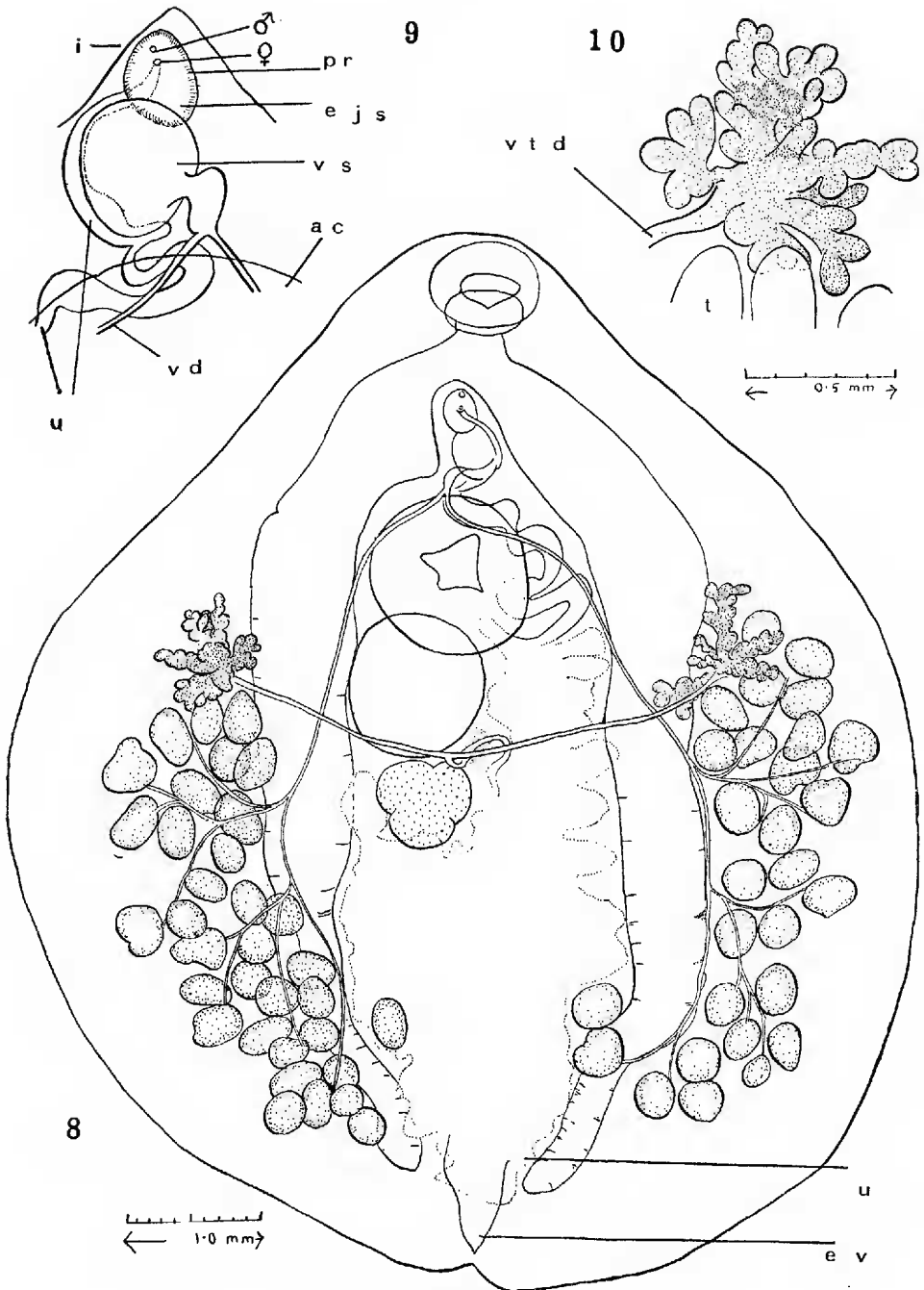
#### THE GENUS NAGMIA.

Nagaty (1930, 107) characterised a new genus, *Nagmia* (type *N. yorkei*) near *Petalodistomum*, but differing from the latter in its greater size, the shape of the vitelline glands, and the greater number of testicular lobules. Nagaty's account does not include any important characters separating the two genera. Difference in size is not of generic value. Our examination of *P. polycladum* indicates that the vitellaria are of the same type. The deeper lobulation of the testes is probably not a generic character. *Nagmia* is, then, to be regarded as a synonym of *Petalodistomum*. *P. yorkei* (Nagaty) from *Trygon* sp., Ceylon, differs from *P. cymatodes* in size, the relative dimensions of the suckers, the extent of the uterine convolutions, relative sizes of receptaculum and ovary, the position and lobulation of the testes, the larger vitellarian tubes and the size of the eggs.

#### PROBOLITREMA ROTUNDATUM T. H. Johnston.

A mounted flattened specimen from the collection of the late Professor S. J. Johnston and collected from an elasmobranch in Sydney, was made available through the kindness of Professor Briggs. It measures 7.8 mm. long by 6.7 mm. wide. The anterior sucker is 0.8 mm. in breadth by 0.7 in length, and the posterior 1.2 mm. in diameter, the ratio thus being 2 : 3. The body form is almost round. The pharynx measures about 0.5 mm. in width. The oesophagus is short, the wide intestinal crura are thrown into a few slight curves and the inner and outer parts of the posterior region show some small closely-set diverticula. The anterior limits of the excretory vesicle are obscured by the uterine folds. The body is minutely scaly.

There are 28 rounded or elliptical testes on one side and 34 on the other. They vary from 0.28 to 0.5 mm. in length, but most of them measure about 0.4 by 0.3 mm. The majority lie extracrucially, but a few partly underlie the crura and a vesicle is intracrucial on each side posteriorly. The vasa efferentia show



Figs. 8-10.

*Probolitrema rotundatum* (specimen from Sydney).—8, ventral view; 9, region of sex apertures, dorsal; 10, a vitellarium, dorsal; a c, acetabulum; e j s, ejaculatory sac; e v, excretory vesicle; i, intestine; p r, prostate; t, testis; u, uterus; v d, vas deferens; v s, vesicula seminalis; v t d, vitelline duct.



that the vesicles are grouped into an anterior, middle and posterior group, though they are arranged to form a continuous field extending from the vitellarium almost to the end of the crura. Some vesicles overlies part of the yolk glands. Each vas deferens travels forwards below the intestine, then inwardly from it, beside the receptaculum on one side and between the uterus and intestine on the other side. The two vasa curve towards the midline just above the anterior edge of the acetabulum and join to form a swollen vesicula seminalis, at first thin-walled, but becoming bent and enlarged into an almost circular, more centrally placed, one. The latter communicates with the thicker-walled ejaculatory sac lying below and in front of it. This sac measures 0.3 by 0.25 mm. The male pore is immediately in front of the uterine aperture and a little distance behind the intestinal bifurcation.

The ovary is slightly trilobed, 0.7 mm. wide by 0.7 mm. long, and just to the right of the midline. The shell gland lies in the angle formed by the receptaculum and ovary. The uterus occupies most of the intercrural region behind the receptaculum. It eventually passes forwards on the left of the latter and the acetabulum. It bends inwardly below the left vas deferens, becoming thrown into a few short folds, then passing to the left of and below the vesicula, then below the ejaculatory sac to the female pore. The receptaculum has a diameter of a millimetre. Eggs measure 0.067 to 0.07 mm. long by 0.052 to 0.055 mm. broad, each having a low rounded knob at one end.

The vitellaria lie extracrurally at the level of the receptaculum and each consists of about six long lobes, each with numerous small rounded sacculations, the whole organ appearing rather compact. The yolk ducts pass inwards and slightly posteriorly, and after travelling between the ovary and receptaculum, join near the shell gland. The common yolk duct forms a curve posteriorly before joining the oviduct as the latter narrows to enter the shell gland.

The species is tentatively assigned to *P. rotundatum* T. H. Johnston, 1934, described recently from the fiddler, *Trygonorrhina fasciata*, from Kangaroo Island. It agrees in body proportions, sucker ratio and in most anatomical details. It differs in the irregular form of the testes and the presence of more marked sacculations on both inner and outer parts of the posterior region of the intestine in *P. rotundatum*.

Another mounted specimen from S. J. Johnston's collection is 4.5 mm. by 3.7 mm. The anterior sucker has a diameter of 0.6 mm., and the acetabulum, 1.1 mm.; ovary, 0.7 mm. broad by 0.56 mm. long; receptaculum, 0.7 to 0.65; testes, 22 and 25, with the diameter ranging between 0.2 and 0.3 mm. Some posterior testes are intercrural. It belongs to the same species as the other Sydney specimen.

A few typical specimens of *P. rotundatum* were taken recently from the peritoneal cavity of a fiddler caught at Port Willunga, South Australia. The flukes were very mobile and the acetabulum very prominent in life, while the intestine showed through as a brownish-yellow structure.

The sucker ratio of this species is similar to that of *P. richiardi* (Lopez, 1888), but the latter is a more elongate parasite with simple crura and with all testes extracrural, while the acetabulum is more anteriorly situated and the vitellaria are relatively more remote in relation to it. The testes are similar in form in the two species, and there are about the same number—24 and 26 being indicated in Looss' figure, but the receptaculum is much smaller and does not reach the acetabulum in the European species.

PROBOLITREMA CLELANDI T. H. Johnston.

Syn. *P. simile* Johnston.

Ofenheim's account (1900) and figure of *P. richiardi*, which Looss (1902) regarded as belonging to a distinct species and named *P. capense*, is not available.

It has the two suckers approximately equal and the acetabulum not especially projecting. The remaining anatomical characters are stated by Looss to be quite similar to those of *P. richiardii* (Lopez). It was collected from *Scyllium* sp. near Capetown.

*P. clelandi* from *Mustelus antarcticus*, South Australia, has a similar sucker ratio, but if Looss' statement is to be taken literally, then it differs from *P. capense* in the form and distribution of the testes, the more posterior position of the acetabulum, the form of the "head lobe," the size of the receptaculum, and the relative position of the ovary which is in front of the mid-length in *P. capense*, where as in *P. clelandi* it is just behind it and behind the level of the vitellaria.

In the original account of *P. simile* it was noted that the species was closely related to, and perhaps identical with, *P. clelandi* from the same host. The sucker ratio given was given as 14 : 17, but some specimens have the two suckers practically equal in diameter. It seems preferable to regard the two species as synonymous, in spite of the fact that the distribution of the testes is different, no vesicles being intercrural in *P. simile*, whereas some are in *P. clelandi*. The species also occurs in *Mustelus antarcticus* in Tasmanian waters.

HOSTS.	PARASITES.	LOCALITY.
<i>Dasyatis kuhli</i> M. & H.	<i>Petalodistomum polycladum</i> <i>Staphylorchis cymatodes</i>	North Queensland " "
<i>Trygonorrhina fasciata</i> M. & H.	<i>Probolitrema rotundatum</i>	Kangaroo Is. and Pt. Willunga, South Australia
<i>Mustelus antarcticus</i> Gunth.	<i>Probolitrema clelandi</i>	Encounter Bay, South Australia; and Tasmania
Host?	<i>Probolitrema rotundatum</i>	Sydney

#### SUMMARY.

The types of *Petalodistomum polycladum* and *P. cymatodes* are re-described. *Nagmia* is a synonym of *Petalodistomum*. *Probolitrema rotundatum* is recorded from new localities and additional information supplied regarding its anatomy. *P. simile* is regarded as a synonym of *P. clelandi*.

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